HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 1 OCTOBER, 1925 No. 8

THE EFFECT OF FEEDING *BACILLUS ACIDOPHILUS*, LACTOSE, DRY SKIM MILK, OR WHOLE MILK ON THE HYDROGEN ION CONCENTRATION OF THE CONTENTS OF THE CECA OF CHICKENS

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INTRODUCTION

The experiments, herein reported, were undertaken to determine in what manner, if any, the hydrogen ion concentration of the cecal contents of chicks would be influenced by feeding them with milk or certain milk products, and the relation of any changes found to occur to the control of coccidiosis.

Why milk feeding should be generally beneficial in intestinal infections of chicks is not made entirely clear by the published work of Rettger and his associates.[4, 5, 6, 7] The experiments of Beach and Corl[1] did not add any information regarding this point, although they proved the practical efficiency of milk in the control of avian coccidiosis. If the reasons for the value of milk feeding in the control of coccidiosis of chicks were known, it might be determined that some of the milk products which can be shipped long distances would be equally effective. In such a case a means of controlling coccidiosis would be available to poultrymen in localities for removed from dairying districts.

These studies have been confined to the ceca because coccidial infection of chicks is usually confined to these parts of the intestinal tract.

NOTE: The writer is indebted to Mr. L. A. van Rooyen for assistance in carrying out much of the detailed work recorded in this paper.

The chickens used in these experiments were confined in cages with grilled bottoms which allowed the droppings to fall onto paper-covered metal trays beneath. The openings in the bottom of the cage were too small to permit the birds to pick at the droppings that collected on the trays. The feed was given in metal cups attached to the grilled doors of the cages. Under these conditions, it was possible to maintain absolute control over all food and other material ingested by the birds.

After some preliminary work, the "spot plate" method was adopted for making the hydrogen ion concentration determinations.* Standard buffer solutions of known pH values at intervals of 0.2 were made according to the formulae of Clark.[3] Methyl red, brom-cresol purple, and brom-thymol blue were the indicators used. In making a pH determination, a small amount of material from the ceca was mixed with a few drops of neutral distilled water in a depression of the porcelain spot plate and one or two drops of indicator added. As a routine procedure, brom-cresol purple was first tried. If the pH was found to be out of the range of this indicator, additional tests were made using brom-thymol blue or methyl red. Comparisons were then made with mixtures of standard buffer solutions and indicator in adjoining depressions until an exact match in color was obtained and the pH value of the cecal material thereby determined.

The study of the flora of the intestines and ceca consisted simply in the microscopic examination of thin smears of the intestinal and cecal contents fixed in methyl alcohol and stained by Gram's method. Differential counts of the Gram-negative and Gram-positive organisms present were made. This count indicated any increase in the relative number of long, slender Gram-positive acidophilus-like rods that occurred.

The Effect of Feeding Cultures of *Bacillus acidophilus*†

The birds used in this experiment had been confined in cages and fed an identical ration for more than thirty days before the experiment was started. It was thought, therefore, that the material in the intestinal tract of each should be quite uniform in character.

Before the culture feeding, as a means of determining the normal pH and flora of the intestinal tract of these birds, six of them were killed and examined. The results are given in table 1.

* The writer is indebted to P. L. Hibbard for valuable advice and assistance in the selection of the method of making hydogen ion concentration determinations.

† The strain of *B. acidophilus* used in the preparation of the milk cultures for these experiments was obtained from Dr. L. F. Rettger.

But slight variation was found in either the pH or the flora of different birds. The pH of the duodenal contents ranged from 6.2 to 6.6, that of the cecal contents from 6.6 to 7.0. The proportion of Gram-positive rods to the total number of bacteria in the duodenal contents varied from 25 per cent to 41 per cent, and in the cecal contents from 32 per cent to 42 per cent.

TABLE 1

pH AND BACTERIAL COUNT OF DUODENAL AND CECAL CONTENTS OF NORMAL FOWLS

Fowl	Section of intestines	pH	Bacterial count
1	Duodenum	6.4	30% Gram+; few forms
	Ceca	7.0	42% Gram+; many forms
2	Duodenum	6.4	25% Gram+; few forms
	Ceca	6.8	32% Gram+; many forms
3	Duodenum	6.4	32% Gram+; few forms
	Ceca	6.8	40% Gram+; few forms
4	Duodenum	6.6	37% Gram+; few forms
	Ceca	6.8	34% Gram+; few forms
5	Duodenum	6.4	41% Gram+; many forms
	Ceca	7.0	41% Gram+; many forms
6	Duodenum	6.2	40% Gram+; many forms
	Ceca	6.6	33% Gram+; many forms

On July 31, 1923, *B. acidophilus* culture feeding was begun. One hundred c. c. of a 48-hour milk culture was given daily to each of twenty-six birds in individual cages. The milk was given in cups suspended on the cage doors. No other drink was allowed until the milk was consumed. The remainder of the diet consisted of whole wheat and cracked yellow corn, the maximum daily consumption of which was seventy-five grams. The milk, therefore, constituted more than half of the food. Starting on the third day and continuing at irregular intervals until the fifty-first day, the birds were killed for examination, one at a time, until twenty had been killed. In all cases, the birds were killed in the morning before the day's allotment of *B. acidophilus* culture had been given. Table 2 gives the detailed results.

No difference between the pH of the duodenal contents of these birds and that of the normal birds was found. The pH of the cecal contents in four birds was 5.6. In the others, it ranged between 6.0 and 7.0. The average pH, therefore, was slightly lower than in the birds that had not received the cultures. However, it was not demonstrated that the pH of the ceca was being progressively lowered since in the last three birds killed on the forty-sixth, forty-ninth and fifty-first days, the pH was 6.8, 6.0 and 6.6, respectively.

TABLE 2

pH and Bacterial Count After the Feeding of *B. acidophilus* Cultures

Fowl	Day killed	Section of intestines	pH	Bacterial count
1	3rd	Duodenum	6.4	96% Gram+; mostly acidophilus-like
		Ceca	7.0	52% Gram+; many acidophilus-like
2	5th	Duodenum	6.4	64% Gram+; few acidophilus-like
		Ceca	7.0	52% Gram+; very few acidophilus-like
3	8th	Duodenum	6.2	60% Gram+; very few acidophilus-like
		Ceca	6.8	64% Gram+; very few acidophilus-like
4	10th	Duodenum	6.4	52% Gram+; very few acidophilus-like
		Ceca	7.0	70% Gram+; many acidophilus-like
5	12th	Duodenum	6.2	62% Gram+; few acidophilus-like
		Ceca	6.6	57% Gram+; few acidophilus-like
6	15th	Duodenum	6.4	51% Gram+; many acidophilus-like
		Ceca	6.4	63% Gram+; many acidophilus-like
7	17th	Duodenum	6.4	100% Gram+; all acidophilus-like
		Ceca	6.8	46% Gram+; mostly acidophilus-like
8	19th	Duodenum	6.4	52% Gram+; 94% acidophilus-like
		Ceca	7.0	70% Gram+; 64% acidophilus-like
9	21st	Duodenum	6.2	50% Gram+; 66% acidophilus-like
		Ceca	5.6	68% Gram+; 99% acidophilus-like
10	23rd	Duodenum	6.4	76% Gram+; 95% acidophilus-like
		Ceca	5.6	65% Gram+; 92% acidophilus-like
11	29th	Duodenum	6.2	93% Gram+; 86% acidophilus-like
		Ceca	6.8	75% Gram+; 75% acidophilus-like
12	31st	Duodenum	6.2	85% Gram+; 83% acidophilus-like
		Ceca	6.6	71% Gram+; 71% acidophilus-like
13	35th	Duodenum	6.6	50% Gram+; 82% acidophilus-like
		Ceca	5.8	60% Gram+; 90% acidophilus-like
14	37th	Duodenum	6.4	70% Gram+; 62% acidophilus-like
		Ceca	6.8	90% Gram+; 88% acidophilus-like
15	39th	Duodenum	6.4	62% Gram+; 42% acidophilus-like
		Ceca	6.2	61% Gram+; 79% acidophilus-like
16	42nd	Duodenum	6.2	75% Gram+; 73% acidophilus-like
		Ceca	6.4	63% Gram+; 66% acidophilus-like
17	44th	Duodenum	6.4	77% Gram+; 61% acidophilus-like
		Ceca	5.6	61% Gram+; 85% acidophilus-like
18	46th	Duodenum	6.4	75% Gram+; 90% acidophilus-like
		Ceca	6.8	70% Gram+; 66% acidophilus-like
19	49th	Duodenum	6.4	80% Gram+; 72% acidophilus-like
		Ceca	6.0	50% Gram+; 86% acidophilus-like
20	51st	Duodenum	6.4	66% Gram+; 75% acidophilus-like
		Ceca	6.6	62% Gram+; 51% acidophilus-like

The differential bacterial count of smears of the duodenal and cecal contents showed an immediate and constant marked increase in the proportionate numbers of Gram-positive organisms, the major portion of which were of the acidophilus type. In the duodenum, the number ranged from 50 per cent to 100 per cent and in the cecum from 46 per cent to 90 per cent. This change in the flora, however, did not increase progressively with the continued feeding of the cultures; in fact, it was more marked in the bird that was killed on the third day than in the one killed on the fifty-first day.

This experiment has demonstrated, therefore, that feeding chickens milk cultures of *B. acidophilus* may cause organisms of the acidophilus type to predominate in the intestinal flora. It has not demonstrated, however, that the acidity of the intestinal contents will be thereby increased. These results are in agreement with those obtained by Rettger and Cheplin[8] in their experiments with rats and human subjects. It does not seem probable, however, that implantation of *B. acidophilus* is an important factor in coccidiosis control unless some other change, such as increase in acidity of the cecal contents, also results. Therefore, in the subsequent experiments, no study of the change in the intestinal flora was made, attention being paid only to changes in hydrogen ion concentration of cecal contents.

Determination of Changes in the Hydrogen Ion Concentration of Cecal Contents by Examination of Cecal Droppings

Browne[2] observed that only a small portion of the material passing through the intestines of a chicken enters the ceca. The coarser material passes directly from the small to the large intestine. A portion of the liquid and finely-divided particles enters the ceca, where it is retained for a considerable time. As a result, the cecal contents consist of a characteristic, homogenous, brown or chocolate colored, pultaceous mass, easily distinguishable from the contents of other portions of the intestines. Browne further observed that the ceca apparently do not continuously discharge into the large intestine but may completely empty themselves periodically after considerable material has accumulated in them. This material passes out in the droppings without becoming mixed with that from other portions of the intestines. Occasionally a dropping consisting entirely of such material from the ceca is passed.

The portion of the droppings coming from the ceca is easily distinguished by its characteristic color and consistency. This suggested the possibility of studying changes in the pH of cecal contents by the examination of the cecal droppings. Such a procedure, if successful, would make observations on the same bird possible as frequently as there were passages of cecal droppings. This would be more satisfactory than the single observation obtained by destroying the bird.

To obtain information on the accuracy of this method, several birds were kept under close observation and killed immediately after a passage of cecal droppings. The pH of the cecal droppings and that of the cecal contents of the same birds were found to be in close agreement, as shown in table 3.

TABLE 3

pH of Cecal Droppings and of the Contents of Different Parts of the Intestines

No. of bird	pH of cecal droppings	pH of different parts of intestines		
		Cecum	Duodenum	Middle of small intestines
19	6.4	6.4	6.2	6.2
22	6.2	6.0	6.2	6.6
13	6.2	6.2	6.2	6.2
12	6.6	6.6	5.6	6.8
14	6.0	6.2	6.2	5.6
17	6.6	6.6	6.4	6.8
4	7.0	6.8	6.0	7.0
5	6.4	6.6	5.8	7.0
18	6.2	6.2	5.8	7.0

This method of determination of the hydrogen ion concentration of the ceca was then applied to five birds remaining from the preceding experiment. Cecal dropping were collected each morning for eleven days. Table 4 gives the results of the pH determinations.

TABLE 4

pH of Cecal Droppings of Fowls Fed *B. acidophilus* Cultures

Fowl No.	pH of cecal droppings									
	Oct. 1	Oct. 3	Oct. 4	Oct. 5	Oct. 10	Oct. 11	Oct. 12	Oct. 13	Oct. 14	Oct. 17
22	7.0	6.0	6.4	6.4	6.4	6.4	7.0	6.8	6.8	6.8
23	—	—	6.4	6.8	6.6	6.6	6.4	—	6.8	6.8
24	—	—	6.4	6.8	6.8	7.0	6.6	6.6	—	7.0
25	6.8	—	—	6.6	6.8	—	6.8	6.8	7.0	—
26	6.8	6.8	6.8	6.8	6.8	7.0	7.0	6.8	7.0	7.0

"—"=No cecal droppings passed.

The limits of variation of the pH of the cecal droppings were 6.0 and 7.0. This is in conformity with the results of hydrogen ion determinations of the cecal contents of the birds in the preceding experiments. The determination of the pH of the cecal contents by this method, therefore, appeared to be accurate and was the procedure adopted for subsequent experiments. Because of the irregularity of the passages and the frequent admixture with urates encountered in the cloaca, it was not always possible to secure suitable samples of cecal droppings from each bird every day. In a large percentage of cases, however, two samples could be secured within twenty-four hours.

EFFECT OF FEEDING CULTURES OF *B. acidophilus* AND LACTOSE

Twenty-five cockerels were used in this experiment. From October 23, 1923, until November 6, each bird was given daily, as a drink, 100 c. c. of 48-hour milk cultures of *B. acidophilus*. This was placed before the birds between 9 o'clock and 10 o'clock each morning. Cultures alone were given for the first four days. During the next ten days, five grams of lactose was added to the milk for each bird. The lactose was then increased to 10 grams for each bird daily for the following nine days. The time required for the consumption of the 100 c. c. of cultures varied with the different birds from one to twenty-four hours. No other drink was allowed until the culture was consumed. Cecal droppings for pH determinations were collected on the morning that culture feeding was begun and on subsequent mornings at intervals of one to five days. In this and in all subsequent experiments, whenever droppings for pH determinations were collected, fresh paper

TABLE 5

pH OF CECAL DROPPINGS OF BIRDS FED DAILY 100 C.C. *B. acidophilus* CULTURES AND 5 GRAMS LACTOSE. LACTOSE INCREASED TO 10 GRAMS ON NOVEMBER 6

Fowl No.	Before feeding	Cultures alone		Cultures+5 grams lactose						
	Oct. 23	Oct. 24	Oct. 26	Oct. 29	Oct. 30	Oct. 31	Nov. 1	Nov. 5	Nov. 9	Nov. 14
1	5.6	6.0	6.0	5.3	4.8	6.6	6.8	5.6	5.4	5.2
2	6.0	6.0	6.8	5.4	5.4	6.4	5.8	5.8	5.4	6.6
3	6.6	6.4	6.4	5.4	6.4	5.8	6.4	5.8	5.6	6.4
4	6.2	6.2	6.2	6.5	—	5.8	5.4	5.6	5.0	6.4
5	6.0	6.2	5.4	—	5.4	5.6	5.6	5.4	—	—
6	6.4	6.8	6.2	6.4	6.4	5.8	6.6	6.8	6.6	4.8
7	6.2	6.0	5.4	6.4	6.6	5.2	6.0	5.0	—	—
8	6.2	6.8	6.2	—	—	—	—	5.8	5.6	5.0
9	5.4	6.8	6.6	5.2	6.0	5.8	5.4	6.6	6.4	5.0
10	6.2	—	6.6	4.8	—	5.6	5.6	5.2	5.0	6.8
11	6.4	6.2	5.4	5.0	5.6	6.8	5.2	—	5.0	6.6
12	6.2	6.2	6.2	4.8	5.8	6.4	5.0	5.0	4.8	6.2
13	6.6	6.4	5.6	4.8	5.8	6.4	5.4	5.4	5.4	5.4
14	6.2	—	—	6.2	6.2	—	—	5.6	4.6	6.2
15	6.4	6.2	5.4	5.6	5.4	6.0	5.8	5.8	6.2	6.0
16	6.6	6.8	6.6	5.8	5.8	6.4	5.4	5.0	6.2	6.0
17	6.2	6.4	6.4	5.6	5.8	6.0	6.4	6.2	5.6	6.8
18	6.2	7.0	5.4	7.0	5.8	7.0	6.8	6.8	—	—
19	6.4	6.4	6.8	5.6	5.4	6.6	5.4	6.2	5.2	4.8
20	6.4	6.4	6.4	6.8	6.8	6.2	5.4	6.0	4.4	—
21	6.6	6.8	5.4	6.6	6.8	—	6.8	6.8	5.2	6.4
22	6.4	—	6.4	6.4	4.8	6.6	5.8	5.4	5.0	6.6
23	6.4	6.8	6.4	4.4	4.8	6.6	5.4	5.4	4.8	—
24	6.8	7.0	6.4	4.8	5.6	6.6	6.6	—	5.4	5.4
25	6.2	—	5.4	5.6	5.8	—	—	—	—	—

"—" = Cecal droppings absent or mixed with other droppings.

was put on the trays. By this means it was known that all cecal droppings collected had been passed since the preceding collection of droppings. The results of the pH determinations appear in table 5.

As in preceding experiments, while the cultures alone were fed, the pH of the cecal droppings was not materially changed. After lactose was added, however, a marked lowering of the pH of the cecal droppings from all birds occurred. In the twenty-five birds the low points were as follows: one, 5.6; seven, 5.4; two, 5.2; six, 5.0; six, 4.8; one, 4.6, and two, 4.4.* The low pH, however, was not constant for the cecal droppings of any individual from day to day. Neither did this change become more marked with the increase of the daily allowance of lactose to 10 grams. The results of this experiment show, therefore, that when chickens are fed daily with cultures of *B. acidophilus* and lactose, acidity of the cecal contents may be produced, but they do not show that the acidity will remain constant during the feeding period.

Variation in the pH of Cecal Droppings During Twenty-four Hours When *B. acidophilus* Cultures and Lactose Are Fed

At this point, it occurred to us that the time of passage of cecal droppings with respect to the time of consumption of the *B. acidophilus* cultures and lactose might be a factor in the variation of the pH of the cecal droppings. Thus, for example, cecal droppings voided in the afternoon and evening, a few hours after the morning feeding of cultures, might be more acid than those voided during the night or morning, twelve or more hours after the cultures were consumed. Information on this point was furnished by a series of three experiments.

In the first experiment, twenty-four of the twenty-five cockerels of the preceding experiment were used. Cups containing 100 c. c. of *B. acidophilus* cultures and 10 grams of lactose were placed before the birds from 9.30 to 11.30 A.M. and then removed. Cecal droppings for pH determinations were collected before 10 o'clock in the morning and again before 5 o'clock in the afternoon. It was not possible to determine whether the droppings collected in the morning had been passed during the previous evening, during the night, or during the earlier morning hours. This was a possible source of error in the pH determinations for these droppings. It was definitely known, however,

* In this paper when reference is made to an abnormal or high acidity, increased, hydrogen ion concentration, low pH, etc., it indicates that the pH of the droppings is between 4.4 and 5.6. By normal acidity or hydrogen ion concentration is meant pH 6.0 to 7.4.

that all droppings collected in the afternoon had been passed since the morning feeding of the cultures and lactose. The pH determinations of the droppings are found in table 6.

TABLE 6

pH OF CECAL DROPPINGS OF COCKERELS FED 100 C.C. OF *B. acidophilus* CULTURES AND 10 GRAMS OF LACTOSE FROM 9:00 TO 11:30 A.M.

Fowl No.	Nov. 15	Nov. 16		Nov. 20		Nov. 21		Nov. 22		Nov. 23		Nov. 24
	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
1	—	5.4	5.0	5.8	—	5.0	—	5.4	—	5.6	5.0	6.2
2	—	5.4	—	—	—	5.2	—	6.2	—	6.4	—	6.4
3	—	5.4	5.2	—	5.2	6.2	—	6.2	—	6.4	5.2	6.2
4	—	6.4	—	5.2	5.2	6.4	—	6.8	—	6.8	5.4	6.4
6	—	6.0	5.0	—	4.8	6.8	5.0	6.8	—	7.0	5.2	6.8
8	—	—	6.0	5.2	—	5.0	5.0	6.0	5.2	5.6	5.0	—
9	—	6.2	5.2	—	5.0	6.2	—	6.4	—	6.2	5.0	6.4
10	5.0	—	5.0	—	5.2	6.8	5.2	6.4	5.0	6.8	5.2	6.8
11	—	—	—	5.8	5.2	5.4	—	6.6	—	7.0	5.4	7.0
12	5.4	—	—	—	5.0	5.0	5.0	5.6	5.4	6.4	—	6.4
13	—	6.2	5.0	6.2	5.4	5.2	—	6.2	5.2	6.0	5.0	6.6
14	—	—	5.0	—	5.2	5.4	—	6.2	5.2	5.4	—	—
15	5.6	5.8	—	—	5.4	6.8	—	6.4	—	6.0	5.2	6.8
16	—	5.8	—	—	5.2	6.6	5.2	6.2	5.4	6.2	5.4	6.6
17	5.4	5.2	5.2	5.0	—	5.2	5.2	5.2	5.2	5.4	5.2	6.4
19	5.2	6.0	5.4	—	5.2	6.0	5.0	5.4	5.0	5.4	5.0	6.4
20	—	—	—	—	—	—	—	—	—	—	—	—
21	—	6.6	—	5.4	—	6.4	—	6.2	—	6.4	5.2	6.8
22	5.6	—	5.2	—	5.2	6.2	5.4	—	5.2	6.2	5.0	6.2
23	5.0	—	5.0	—	—	5.0	4.8	5.0	—	—	—	—
24	—	—	—	—	5.0	5.0	5.0	—	—	6.4	—	—

"—"=Cecal droppings absent or mixed with other droppings.

A study of this table shows that with one exception (No. 8 on November 16, pH 6.0), the pH of all cecal droppings collected in the afternoon fell between 4.8 and 5.4. The droppings collected in the morning, however, showed the same pH variation as previously observed, slightly more than half being between 6.0 and 7.0 and the remainder between 5.0 and 6.0. It was noted that the afternoon passages of cecal droppings, the pH of which was low, were quite liquid or filled with gas bubbles. In this condition they quickly became mixed with other droppings on the trays which made it impossible to secure satisfactory samples in many instances.

Failures to secure both morning and afternoon samples of cecal droppings from the same bird, therefore, were frequent, but this was accomplished in forty-three instances. In twenty-six instances, the pH of the morning droppings was between 6.0 and 7.0 and that of the afternoon droppings from the same bird was from 0.8 to 1.8 lower or between 5.0 and 5.4. In the remaining seventeen instances, the

difference in the pH was not marked, all of the morning determinations falling between 5.0 and 5.8 and the afternoon determinations between 4.8 and 5.4. In no case, however, was the pH of a morning dropping lower than that of an afternoon dropping from the same bird.

The procedure of feeding cultures of *B. acidophilus* and lactose and collecting cecal droppings twice daily for pH determinations was next applied to fifteen hens one year old. The pH determinations are recorded in table 7.

TABLE 7

pH OF CECAL DROPPINGS OF HENS GIVEN 100 C.C. OF *B. acidophilus* CULTURES, AND 10 GRAMS OF LACTOSE, FROM 9:00 TO 11:30 A.M.

Bird No.	Dec. 5		Dec. 6		Dec. 7		Dec. 10		Dec. 11	
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.
26	6.0	5.2	6.4	5.4	6.6	5.2	5.4	5.2	5.8	5.0
27	6.4	5.0	6.4	5.0	6.2	5.0	6.0	5.0	—	5.2
28	6.4	5.2	6.6	5.2	6.8	—	6.8	—	6.4	—
29	6.4	—	6.4	5.0	6.6	5.2	5.6	5.2	5.4	5.0
30	6.4	—	6.6	5.2	6.4	5.4	5.8	5.6	6.0	5.2
31	6.4	—	6.0	5.0	—	5.4	5.2	5.2	5.4	5.4
32	6.0	5.2	6.4	5.2	6.2	5.0	5.0	5.0	5.4	5.0
33	6.2	5.4	6.4	5.0	6.2	—	—	—	6.2	—
34	6.8	—	6.0	5.0	6.6	5.2	—	5.2	5.4	5.4
35	6.6	5.0	6.6	5.2	6.4	5.2	5.2	5.0	6.2	5.2
36	6.2	5.6	6.6	5.4	6.4	5.0	6.6	4.8	6.4	5.2
37	6.4	5.2	6.0	—	6.2	5.0	6.2	5.0	6.2	—
38	6.4	5.4	6.6	—	6.4	5.2	5.4	5.2	6.6	5.2
39	6.2	—	6.2	5.2	6.0	5.2	6.2	—	5.4	—
40	6.2	5.2	6.2	5.0	6.4	5.0	—	5.0	—	—

"—"=Cecal droppings absent or mixed with other droppings.

This table shows the results to correspond closely to those of the preceding experiment. The pH of the cecal droppings collected in the afternoon was uniformly between 5.0 and 5.4, while that of a majority of the samples of droppings collected in the morning was between 6.0 and 6.8.

The same fifteen yearling hens were used in the third experiment. Between 9 and 10 o'clock each morning, 50 c. c. of *B. acidophilus* milk culture and 5 grams of lactose were introduced into the crop of each bird with a pipette. This insured a uniform dose of cultures and lactose for each bird at a given time each day. Cecal droppings were collected and pH determinations were made twice daily as before. The pH determinations are given in table 8.

From this table it is seen that the pH of the cecal droppings, passed within six hours after the administration of 50 c. c. of *B. acid-*

ophilus cultures and 5 grams of lactose, was in every instance between 4.8 and 5.4. The pH of the cecal droppings passed from eight to twenty-four hours after the administration of the milk cultures and lactose, however, was in most instances above 6.0.

The results of this series of three experiments demonstrate that when milk cultures of *B. acidophilus* and lactose are fed to chickens, the acidity of the cecal contents, as indicated by hydrogen ion concentration determinations, increases within a few hours. This change, however, is of short duration as is shown by pH determinations made on the cecal droppings passed eight to twenty-fours hours after the *B. acidophilus* cultures and lactose had been fed.

TABLE 8

pH of Cecal Droppings of Hens Given 50 c.c. of *Bacillus accidophilus* Cultures and 5 Grams of Lactose with a Pipette

Bird No.	Dec. 12		Dec. 13		Dec. 14		Dec. 15		Dec. 18		Dec. 19
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
26	6.2	5.2	6.6	4.8	5.6	5.0	—	—	6.0	—	—
27	6.0	5.2	6.2	4.6	6.2	5.0	—	—	—	5.0	—
28	6.8	5.4	6.8	—	6.4	5.0	6.8	—	6.0	—	—
29	6.6	5.0	6.4	5.2	6.4	5.0	6.4	—	5.6	5.2	6.4
30	6.2	5.2	6.0	5.2	6.6	5.4	5.6	—	6.2	4.8	—
31	6.0	5.2	6.2	5.2	6.0	5.4	6.2	—	5.8	4.8	6.4
32	5.6	4.8	6.0	5.0	5.6	5.2	—	—	—	4.6	5.8
33	5.8	4.8	6.2	5.0	6.2	4.8	5.4	—	6.4	—	—
34	5.4	5.4	5.8	5.4	5.6	4.8	6.4	—	5.8	—	6.0
35	6.4	5.2	5.8	5.2	6.2	5.2	6.0	—	6.4	5.4	—
36	—	5.2	6.2	5.2	6.4	5.6	—	—	6.4	5.2	—
37	6.2	5.0	6.0	5.0	6.4	4.8	6.4	—	6.2	5.0	—
38	6.4	4.8	6.8	5.2	6.6	4.8	6.6	—	6.4	5.0	—
39	5.6	5.2	5.8	5.0	6.2	5.2	6.0	—	6.4	4.8	—
40	6.2	5.2	—	—	6.4	—	—	—	6.2	5.2	—

"—"=Cecal droppings absent or mixed with other droppings.

Effect of the Oral Administration of Lactose Alone

This experiment was designed to determine if by feeding chickens lactose alone the hydrogen ion concentration of the cecal contents would be changed to the same extent as when milk cultures of *B. acidophilus* were fed alone or combined with lactose. Ten yearling hens, divided into two groups of five each, were used. Each bird in one group received 5 grams of lactose each morning. In the other group, 5 grams of lactose was given to each bird morning and afternoon. The purpose of this was to determine whether by two feedings, the pH of the cecal contents would remain continuously low instead of for a few

hours only after feeding, as occurred when *B. acidophilus* cultures and lactose were fed in the morning only.

Beginning on the day the first dose of lactose was given, cecal droppings for pH determinations were collected on five successive days. The pH determinations are given in table 9.

TABLE 9

pH of Cecal Droppings of Hens Given One or Two 5-Gram Doses of Lactose Daily

Bird No.	Jan. 28		Jan. 29		Jan. 30		Jan. 31		Feb. 1		Feb. 2
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
One 5-gram feeding of lactose daily											
34	6.4	—	5.6	5.2	6.0	—	5.4	5.2	6.0	5.6	6.0
39	6.6	—	6.8	—	6.4	—	6.6	—	6.8	—	7.0
40	6.8	—	6.6	5.4	—	—	6.6	—	6.4	—	—
41	—	5.6	6.6	—	6.8	5.4	6.4	—	5.6	—	6.6
42	6.0	—	—	—	5.4	5.4	6.0	—	6.0	—	6.2
Two 5-gram feedings of lactose daily											
26	6.8	—	5.2	5.4	5.0	—	5.2	—	5.2	—	5.6
27	6.4	—	—	—	5.0	—	—	—	—	—	—
28	7.0	—	—	—	—	5.0	5.6	—	5.6	—	5.2
32	6.8	—	—	—	—	—	—	—	5.0	—	—
33	—	5.2	—	—	5.4	5.4	5.6	—	5.4	—	5.2

"—" = Cecal droppings absent or mixed with other droppings.

TABLE 10

A Reversal of the Groups in Table 9.

Bird No.	Feb. 4		Feb. 5		Feb. 6		Feb. 7		Feb. 8		Feb. 9
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
One 5-gram feeding of lactose daily											
26	6.2	5.6	6.6	—	6.6	—	7.0	—	—	—	6.4
27	6.6	—	5.8	—	5.2	5.2	5.4	—	6.0	5.4	5.8
28	6.8	—	5.6	—	6.8	—	6.8	4.8	—	5.6	6.0
32	5.8	—	—	—	6.2	—	—	5.2	6.6	—	6.4
33	6.2	5.8	—	5.2	7.0	—	6.2	—	—	5.0	5.8
Two 5-gram feedings of lactose daily											
34	6.8	—	5.0	5.0	5.0	—	5.0	—	—	—	—
39	6.4	—	5.2	—	—	—	—	—	5.0	—	—
40	6.2	—	—	—	5.2	—	5.6	—	—	—	—
41	6.6	—	5.0	5.0	5.2	5.0	4.8	—	5.0	—	5.0
42	6.0	—	5.4	—	5.0	5.0	5.0	—	4.8	5.2	—

"—" = Cecal droppings absent or mixed with other droppings.

The watery and gaseous condition of the cecal droppings from these birds seemed even more pronounced than when birds were fed *B. acidophilus* cultures. As a result, failures to obtain suitable samples of cecal droppings were frequent.

The pH determinations of cecal droppings from the birds given lactose in the morning only were uniformly below 5.6 when collected in the afternoon, but, with few exceptions, were between 6.0 and 6.8 when collected in the morning. These results are in accordance with those obtained when *B. acidophilus* cultures, in addition to lactose, were fed. When lactose was given twice daily, however, with the exception of those collected on the morning of the first day before any lactose had been fed, the pH of both morning and afternoon cecal droppings ranged from 5.0 to 5.6.

This experiment was now repeated. The only change of procedure was a reversal of the two groups with respect to lactose administration. The birds in the group that formerly received one daily 5-gram dose of lactose now received two, and those in the group that formerly received two doses of lactose now received one. The purpose was to determine whether the uniform acidity of the cecal droppings, passed by the birds to which lactose was administered twice daily, was due to the administration of lactose and not to a peculiarity of the birds. The pH determinations are recorded in table 10.

These results are in accordance with those of the preceding trial. When the birds were given a 5-gram dose of lactose in the morning only, the pH determinations of the cecal droppings passed during the afternoon were between 5.0 and 5.6, while those of the cecal droppings passed during the night or in the morning ranged, with few exceptions, from 6.0 to 7.0. When the birds received 5-gram doses of lactose both morning and afternoon, however, the limits of variation of the pH of both the morning and afternoon collections of cecal droppings were determined to be 4.8 and 5.6.

The results of the three trials indicate that the hydrogen ion concentration of the cecal contents of chickens can be increased as readily and to an equal degree by the administration of 5 grams of lactose alone as by giving 5 grams or 10 grams of lactose plus 50 c. c. or 100 c. c. of milk cultures of *B. acidophilus*. The results also suggest that the hydrogen ion concentration of the cecal contents of a chicken that is given two 5-gram doses of lactose each day will be continuously greater than that of birds fed grain only.

EFFECT OF FEEDING MASH CONTAINING LACTOSE OR DRY SKIM-MILK

In the preceding experiment, it was found that a single oral administration of 5 grams of lactose would result in lowering the pH of the cecal contents. Within less than twenty-four hours, however, the cecal contents would again show a normal pH. When two 5-gram doses of lactose were given at an interval of about eight hours, the pH of the cecal contents appeared to remain continuously lowered. It seemed probable, therefore, that a low pH in the ceca could be more readily maintained if means were provided for a more or less continuous flow of lactose through the intestinal tract. It was thought that this could be effectively accomplished by mixing, with the food, lactose or some

TABLE 11

PH OF CECAL DROPPINGS OF HENS FED DAILY 40 GRAMS OF MASH CONTAINING 5 PER CENT OF LACTOSE

Hen No.	Feb. 6		Feb. 7		Feb. 8		Feb. 9		Feb. 10		Feb. 11		Feb. 12		Feb. 13		Feb. 14	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1	6.6	6.2	6.0	5.8	6.4	—	6.6	—	6.2	—	6.6	5.2	6.6	—	5.8	5.4	5.6	5.2
2	—	—	5.2	—	6.8	—	5.6	—	6.4	5.2	5.6	5.2	6.8	5.8	6.2	5.4	6.6	6.8
3	—	—	—	6.2	—	—	6.6	—	6.6	—	6.6	6.0	6.6	—	5.0	5.2	6.8	6.6
4	6.8	5.2	5.4	5.2	6.4	5.0	6.2	—	6.0	—	5.4	6.2	6.4	5.0	5.6	—	5.6	5.2
5	—	—	6.0	—	—	—	5.8	—	6.6	—	6.6	—	7.0	6.0	6.8	5.4	5.6	—

"—"=Cecal droppings absent or mixed with other droppings.

other dry milk product, such as dry skim-milk, which contains a large percentage of lactose. Dry skim-milk was thought to be particularly worthy of trial because of the suitability, as a food for poultry, of the ingredients it contains in addition to lactose. This composition, it was believed, would make its employment more practicable than that of lactose for field use in the control of coccidiosis in case it was found that lactose feeding was effective against the disease.

A series of four feeding trials was conducted therefore, to determine whether feeding mash containing lactose or dry skim-milk would cause the pH of the ceca to remain continuously lowered, and if so, what proportion of the total food consumption should be lactose or dry skim milk in order to bring about this change.

In the first trial, 40 grams of mash containing 5 per cent of lactose were fed daily to each of five yearling hens. Mash only was available to the birds from 10 a.m. to 4 p.m., but at other times mixed whole grain was also before them.

TABLE 12

pH OF CECAL DROPPINGS OF CHICKENS FED MASH CONTAINING 10 PER CENT LACTOSE (GROUP I) OR 20 PER CENT DRY SKIM MILK (GROUP II). FEEDING STARTED ON FEBRUARY 26

	Fowl No.	Feb. 26		Feb. 27		Feb. 28		Feb. 29		Mar. 1	Mar. 3		Mar. 4		Mar. 5		Mar. 6		Mar. 7		Mar. 8
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.
GROUP I	6	6.6	5.4	5.8	5.0	6.2	5.0	6.6	5.2	6.0	6.2	—	6.2	5.0	6.2	4.8	5.8	—	6.4	5.2	6.0
	26	—	5.2	6.8	5.0	6.8	6.0	6.2	5.8	6.8	6.4	5.0	6.4	6.2	6.4	5.2	6.8	5.0	6.6	5.0	6.8
	1	6.6	5.6	6.6	—	5.6	—	5.8	—	6.6	6.4	—	6.8	—	6.8	—	6.4	—	6.6	—	6.6
	2	5.6	5.4	6.4	—	5.4	—	5.4	—	5.4	5.4	—	5.0	—	6.0	—	—	5.2	6.4	—	6.6
GROUP II	4	6.8	5.4	6.6	4.8	6.8	4.8	5.6	5.6	6.6	6.8	—	5.8	6.4	—	—	5.4	—	7.0	5.4	6.8
	7	6.6	6.4	—	5.8	6.2	—	5.0	5.0	—	4.8	—	6.0	6.0	—	—	6.0	5.0	5.4	—	—
	8	5.0	5.6	5.6	—	5.0	—	5.6	5.6	5.4	6.2	—	6.2	6.0	—	—	6.0	—	6.2	—	—
	34	—	—	—	—	—	—	—	—	—	6.2	—	6.6	5.8	—	—	5.0	—	6.0	—	5.0

"—"=Cecal droppings absent or mixed with other droppings.

Cecal droppings for pH determinations were collected between 8:30 and 9:00 a.m. and between 4:00 and 4:30 p.m. for nine successive days. The results, as recorded in table 11, show that, while in many instances the pH of the cecal droppings was found to be between 5.0 and 5.6, this occurred in less than half of the pH determinations. It occurred with greater frequency in the afternoon droppings than in those collected in the morning. This shows that acidity of the ceca can be increased by feeding hens lactose mixed with mash, but indicated that the amount used in this case was too small.

In the second trial, two groups of four hens each were used. To Group I was fed mash containing 10 per cent of lactose, and to Group II mash containing 20 per cent of dry skim milk. The dry skim milk contained 50.6 per cent of lactose, which made the lactose content approximately the same in the mash for both groups. The amount consumed by each bird was determined by placing a weighed amount in the feed cups each morning and weighing out the unconsumed portion on the following morning. Cecal droppings for pH determination were collected twice daily in the first trial. The results are given in table 12.

It was found that in neither group was there a constant increase of acidity in the cecal contents. The pH of droppings collected in the afternoon was, with few exceptions, below 5.6, but in most instances the pH of the morning collection of night droppings was determined to be between 6.0 and 7.0.

The daily mash consumption by the individual birds varied from 45 to 120 grams. The daily lactose consumption by the individuals, therefore, varied from 4.5 to 12 grams in Group I and from 2.25 to 6 grams in Group II. The cecal droppings passed during the night following a day of heavy mash consumption frequently, but not uniformly, had a low pH value. It would appear, therefore, that to maintain the hydrogen ion concentration of cecal contents of chickens, constantly greater than that of birds fed entirely with grain, the food, irrespective of the amount consumed, must contain more than 10 per cent of lactose.

In the third trial with four hens, the percentage of lactose in the mash was increased to twenty. Cecal droppings for pH determinations were collected twice daily as before. The results of pH determinations are given in table 13.

By this table, it is seen that the pH values of all cecal droppings were between 4.8 and 5.6. These results, indicate, therefore, that an abnormal degree of acidity can be continuously maintained in the ceca of chickens when 20 per cent of the food is lactose.

TABLE 13

pH of Cecal Droppings and Food Consumption of Hens Fed Mash Containing 20 per cent Lactose. Feeding Started on March 18, 1924

Fowl No.	March 18		March 19		March 20		March 21		March 22		Mar. 23
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
1	6.4	—	5.4	4.8	5.0	—	4.8	5.0	4.8	4.8	5.0
2	6.8	—	5.6	4.8	4.8	5.0	4.8	4.8	5.0	5.2	5.0
4	6.8	—	4.8	5.2	4.8	5.2	4.8	5.2	5.2	—	5.2
42	6.2	—	4.8	—	—	—	—	—	5.4	—	5.0

Grams of mash consumed

Fowl No.	March 18	March 19	March 20	March 21	March 22	Mar. 23
1	65	60	55	50	50	
2	100	85	100	100	90	
4	105	70	60	70	90	
42	110	70	70	65	30	

Lactose—equivalent in grams

Fowl No.	March 18	March 19	March 20	March 21	March 22	Mar. 23
1	13	12	11	10	10	
2	20	17	20	20	18	
4	21	14	12	14	18	
42	22	14	14	13	6	

"—" = Cecal droppings absent or mixed with other droppings.

In the last of the series of four feeding trials, mash containing 30 per cent of dry skim milk was fed to five hens. The pH values of the cecal droppings were determined twice daily and are recorded in table 14:

TABLE 14

pH of Cecal Droppings and Food Consumption of Hens Fed Mash Containing 30 per cent Dry Skim Milk. Feeding Started March 24, 1924

Fowl No.	March 25		March 26		March 27		March 28		March 29		Mar. 30
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
41	6.6	5.6	5.0	5.0	6.6	5.6	6.6	—	5.2	6.0	5.4
42	5.2	5.2	6.0	5.2	6.6	5.2	5.0	—	5.2	5.0	6.2
43	5.0	—	5.0	5.4	5.2	5.0	5.0	5.0	5.0	5.0	6.2
44	5.6	5.4	5.8	5.2	6.4	5.0	5.2	5.0	5.4	5.0	5.4
45	—	—	7.0	5.6	6.8	—	6.6	—	6.4	—	—

Grams of mash consumed

Fowl No.	March 25	March 26	March 27	March 28	March 29	Mar. 30
41	35	90	95	85	100	
42	60	45	75	90	120	
43	80	70	70	55	70	
44	70	95	95	95	100	
45	65	80	45	60	45	

"—" = Cecal droppings absent or mixed with other droppings.

In this case, all of the cecal droppings collected in the afternoon and many of the morning samples showed a pH of less than 5.6. The pH of morning samples, however, was frequently between 6.0 and 7.0. These results show that the amount of dry skim milk was too small. It seems reasonable to assume that had 40 per cent instead of 30 per cent dry skim milk been used, the results would have corresponded to those obtained with mash containing 20 per cent lactose.

The Rapidity of the Development and the Duration of the Increase in the pH of the Cecal Contents of Chickens Produced by a Single Feeding of Miscellaneous Milk Products.

These tests were designed to show the comparative effectiveness of sweet whole milk and the milk products that were used in the experiments discussed in preceding pages in lowering the pH values of the cecal contents of chickens. They were also expected to indicate the length of the time that acidity in the ceca produced by a single feeding would persist and thereby assist in determining the frequency with which feedings should be given in order to maintain the acidity in the ceca continuously. Individual birds were fed varying amounts of the different products and were killed at varying intervals afterward. The effect on the pH of the cecal contents was determined by comparing the pH determinations of the cecal droppings passed before feeding with those of the cecal contents after death. The number of birds used, the products fed, and the elapsed time between feeding and killing were as follows:

12 birds were fed from 50 c.c. to 75 c.c. of sweet whole milk and killed in from 1 to 2½ hours.

1 bird was fed 100 c.c. of milk cultures of *B. acidophilus* and killed in 2¼ hours.

9 birds were fed 50 c.c of milk cultures of *B. acidophilus* and 5 grams of lactose and killed in from 2 to 24 hours.

23 birds were fed 2 to 4 grams of lactose and killed in from 2 to 17 hours.

The results are recorded in table 15.

Summarizing the results as shown in the table, we find that:

No change had occurred in the ceca of the three birds killed in from 1 to 1¾ hours after they were fed from 50 to 75 c.c. of sweet whole milk.

Acidity had developed in the ceca of fifteen of nineteen birds killed in from 2 to 2½ hours after being fed 75 c.c. of sweet whole milk

TABLE 15

pH OF CONTENTS OF CECA OF CHICKENS KILLED AT VARYING INTERVALS AFTER ADMINISTRATION OF MISCELLANEOUS MILK PRODUCTS

	Age and sex of birds	Milk product	Amount	Time between feeding and killing	pH of cecal droppings before feeding	pH of cecal contents after death	
						Right	Left
32	Mature hen	Sweet whole	60 c.c.	1 hr.	6.0	6.0	6.0
43	Cockerel	Sweet whole	75 c.c.	1½ hrs.	6.8	6.0	6.0
27	Mature hen	Sweet whole	50 c.c.	1½ hrs.	7.0	6.4	6.4
34	Mature hen	Sweet whole	75 c.c.	2 hrs.	6.0	5.0	5.0
144	Cockerel	Sweet whole	75 c.c.	2 hrs.	6.8	6.6	6.4
145	Cockerel	Sweet whole	75 c.c.	2 hrs.	6.2	6.0	6.0
146	Cockerel	Sweet whole	75 c.c.	2 hrs.	6.6	5.4	5.6
147	Cockerel	Sweet whole	75 c.c.	2 hrs.	6.0	5.0	5.0
148	Cockerel	Sweet whole	75 c.c.	2½ hrs.	6.8	6.0	6.0
149	Cockerel	Sweet whole	75 c.c.	2½ hrs.	6.0	5.2	5.4
410	Cockerel	Sweet whole	75 c.c.	2½ hrs.	6.8	5.0	5.0
24	Mature hen	Sweet whole	75 c.c.	2½ hrs.	6.8	5.0	5.4
40	Mature hen	Acidophilus culture	100 c.c.	2½ hrs.	6.6	5.0	5.4
20	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	2 hrs.	6.0	5.0	
16	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	2 hrs.	6.4	4.8	
4	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	4 hrs.	6.0	5.2	
6	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	4 hrs.	6.2	5.2	
3	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	6 hrs.	6.0	5.0	
9	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	6 hrs.	6.0	5.2	
11	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	8 hrs.	6.6	5.4	
10	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	24 hrs.	6.8	6.2	
15	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	24 hrs.	5.8	6.6	
50	Cockerel	Lactose	4 gms.	2½ hrs.	6.6	5.4	5.6
51	Cockerel	Lactose	4 gms.	2½ hrs.	6.4	5.6	5.0
52	Cockerel	Lactose	4 gms.	2½ hrs.	6.4	5.4	5.6
53	Cockerel	Lactose	4 gms.	2½ hrs.	6.6	5.6	5.4
54	Cockerel	Lactose	4 gms.	2½ hrs.	6.0	5.0	5.4
55	Cockerel	Lactose	4 gms.	2½ hrs.	6.0	5.6	5.4
56	Cockerel	Lactose	4 gms.	2½ hrs.	6.6	5.8	6.0
101	Cockerel	Lactose	4 gms.	8½ hrs.	6.2	5.4	5.6
102	Cockerel	Lactose	4 gms.	8½ hrs.	6.4	5.2	5.4
103	Cockerel	Lactose	4 gms.	8½ hrs.	6.6	5.0	5.0
141	Cockerel	Lactose	4 gms.	8½ hrs.	6.8	5.4	5.8
142	Cockerel	Lactose	4 gms.	8½ hrs.	6.8	5.6	6.2
170	Cockerel	Lactose	4 gms.	8½ hrs.	6.4	5.2	5.4
64	Cockerel	Lactose	2 gms.	12 hrs.	6.0	5.4	5.6
66	Cockerel	Lactose	2 gms.	12 hrs.	6.0	6.4	6.0
67	Cockerel	Lactose	2 gms.	12 hrs.	6.6	6.6	7.0
70	Cockerel	Lactose	2 gms.	12 hrs.	6.4	6.4	6.2
41	Cockerel	Lactose	4 gms.	12 hrs.	6.2	5.2	5.2
42	Cockerel	Lactose	4 gms.	12 hrs.	6.6	5.0	5.8
44	Cockerel	Lactose	4 gms.	12 hrs.	6.6	5.0	5.6
22	Cockerel	Lactose	4 gms.	12 hrs.	6.8	6.4	5.2
45	Cockerel	Lactose	4 gms.	12 hrs.	6.6	6.6	6.2
1	Cockerel	Lactose	4 gms.	17 hrs.	6.8	5.6	6.8
2	Cockerel	Lactose	4 gms.	17 hrs.	6.2	6.6	6.4

100 c.c. of 48-hour milk cultures of *B. acidophilus*, 50 c.c. of milk cultures of *B. acidophilus* plus 5 grams of lactose, or 4 grams of lactose.

The acid condition was still present in two birds killed in six hours and in two killed in eight hours after being fed 50 c.c. of *B. acidophilus* cultures plus 5 grams of lactose; in five of six birds killed in 8½ hours after they were fed 4 grams of lactose; in one of four birds killed in 12 hours after being fed 2 grams of lactose; and in three of five birds killed in 12 hours after a feeding of 4 grams of lactose.

The pH values of the ceca of two birds killed 24 hours after the feeding of 50 c.c. of *B. acidophilus* cultures plus 5 grams of lactose, and of two others killed 17 hours after the feeding of 4 grams of lactose was that of normal cecal contents.

An important point not shown in the table is that cecal droppings of the birds killed in from eight to twenty-four hours after the feeding of a milk product showed a pH value lower than that found in the cecal droppings passed prior to the feeding. This is mentioned to show that when the pH values of the cecal contents of the birds that were killed in from 8½ to 24 hours after a feeding were found to be normal, it could be interpreted as a return to normal (6.0 to 7.4) from a lower point, not as a failure of the treatment to produce acidity in the ceca of the birds.

In most instances the pH values of both ceca of the same bird were in close agreement. Exceptions to this were found in birds Nos. 142, 22 and 1, in which the pH of the contents of the two ceca were 5.6 and 6.2, 6.4, and 5.2, and 5.6 and 6.8, respectively. This variation in the character of the contents of the two ceca of the same bird may be a source of occasional error in the interpretation of the pH value of cecal droppings as representing those of cecal contents of a bird.

The tests demonstrate, therefore, that a certain abnormal degree of acidity may be produced in the ceca of chickens within two hours after feeding suitable amounts of sweet whole milk, milk cultures of *B. acidophilus* or lactose. The acidity so produced may persist for from eight to twelve hours, but probably not for a longer period.

Summary and Conclusions

Feeding milk cultures of *B. acidophilus* to chickens resulted in the implantation of *B. acidophilus* in the ceca. In some instances, nearly 100 per cent of the bacteria present in smears of the cecal contents stained by Gram's method were of the acidophilus type. The implantation of *B. acidophilus* in the ceca of chickens, however, did not change the pH value of the cecal contents.

The part of the droppings of chickens originating in the ceca are voided separately and can be differentiated from the part of the droppings from other portions of the intestinal tract. It is possible, therefore, to study changes in the cecal contents of the same chicken that occur from day to day.

The pH of the cecal contents of chickens was changed from the normal range of 6.0 to 7.4 to a range of 4.4 to 5.6 by feeding sufficient amounts of whole sweet milk, milk cultures of *B. acidophilus*, milk cultures of *B. acidophilus* plus lactose, lactose alone, or dry skim milk.

Since lactose is the only ingredient common to all of the milk products used, the change in hydrogen ion concentration of the cecal contents produced by feeding milk or a milk product would appear to be due to the lactose it contains.

The change in the hydrogen ion concentration of cecal contents from a single feeding of a milk product occurred within two to two and one-half hours after the feeding and returned to normal within eight to twenty-four hours after the feeding. The rapidity of development and the short duration of the change in hydrogen ion concentration indicates that it is not a result of modification of the flora of the intestinal tract.

An abnormal degree of acidity in the ceca was constantly maintained by the individual administration to chickens of one or two grams of lactose twice each day at an interval of about eight hours, or by the continuous feeding of mash mixtures containing 20 per cent of lactose. The feeding of mash containing 40 per cent dry skim milk would also provide approximately 20 per cent of lactose in the mash and should, therefore, accomplish the same result.

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THE INFLUENCE OF FEEDING LACTOSE OR DRY SKIM MILK ON ARTIFICIAL INFECTION OF CHICKS WITH *EIMERIA AVIUM*

J. R. BEACH AND D. E. DAVIS

INTRODUCTION

The experiments reported in this paper consist of a series of five trials in which it was attempted to combat artificially-produced coccidial infection in chicks by feeding them with sufficient lactose or dry skim milk to change the hydrogen ion concentration of the ceca from the normal range of 6.0–7.4 to a range of 4.4–5.6. It was thought that, by this means, an environment unfavorable or destructive to the tissue-invading stages of the parasite, viz., the sporozoites and merozoites, might be created.

The first three trials were carried out under laboratory conditions, the chicks being confined in cages with grilled bottoms and fed in cups suspended on the cage doors. In the last two trials, the chicks were reared in brooder pens under normal field conditions, except that no outside runs were provided.

After the feeding of lactose or dry skim milk was begun, the chicks were inoculated by introducing into their crops with a pipette a large number of sporulated oöcysts of *Eimeria avium*. A control group of chicks that was fed neither lactose nor dry skim milk was included in each trial. An estimate of the number of cysts administered to each chick was obtained by making a direct microscopic count of the cysts in $\frac{1}{100}$ c.c. of the inoculum. Material for inoculation was provided by cultures of the cecal contents of chicks affected with coccidiosis prepared as follows: A thin layer of cecal contents containing large numbers of oöcysts was spread over the surface of salt solution agar plates.[1] Salt solution to keep the surface of the plates moist was added as required. The cultures were incubated at room temperature until microscopic examination showed that sporulation of the oöcysts had occurred.

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[1] The writers are indebted to H. W. Graybill for suggesting the use of and furnishing the formula for the "salt solution agar." The formula is as follows: Agar, 20 gms.; sodium chloride, 5 gms.; distilled water, 1000 c.c. The agar is cut up, tied in a gauze bag and washed for two hours in running water before the medium is made up.

TABLE 1

pH of Cecal Droppings and Effect of Inoculation with Sporulated Oöcysts in First Trial

	Fowl No.	Mar. 15		Mar. 16		Mar. 17		Mar. 18		Mar. 19		Mar. 20		Mar. 21		Mar. 22		Mar. 23		Mar. 24		Mar. 25	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Group I (Lactose)	51	5.4	5.6	—	—	5.4	—	X	—	—	5.6	—	X	—	5.6	—	—	—	—	X	5.0	5.2	—
	52	—	—	5.6	—	X	—	5.6	5.6	—	—	5.6	—	B	—	5.6	—	—	—	—	—	—	—
	53	5.6	4.8	—	—	X	—	5.4	5.2	—	5.0	5.0	5.0	—	—	5.0	—	—	—	5.2	5.2	—	—
	54	—	4.8	—	—	X	—	X	—	—	—	5.6	5.4	B	—	B	—	B	—	—	—	—	—
	55	5.4	5.2	5.4	—	5.0	—	5.0	5.4	5.6	—	5.2	5.2	5.4	5.4	5.2	—	5.4	—	—	5.4	5.4	5.2
Group II (Controls)	56	6.0	6.0	5.6	—	5.8	—	5.6	5.6	5.8	—	—	—	B	B	B S	S	S	S	D			
	57	6.2	5.8	6.2	—	6.4	—	6.2	—	6.2	6.0	B	B S	D									
	58	6.2	—	6.0	—	6.0	—	6.0	—	5.4	—	B	B S	B S	D								
	59	6.8	—	6.6	—	6.4	—	6.6	—	6.0	5.4	B	B S	D									
	60	6.4	—	—	—	6.0	—	—	—	—	—	—	—	B	B	B	—	—	—	—	—	—	—

S = Bird visibly sick.
B = Cecal droppings contain blood.
D = Died from coccidiosis.
X = Cecal droppings watery and mixed with other portion of droppings.
— = No cecal droppings.

First Trial

Ten chicks, four weeks old, were divided into two groups of five. They had been reared in an environment thought to be free from *Eimeria avium*. From March 14, 1924, each bird in Group I was given 1 gram of lactose twice daily at 9 a. m. and 4:30 p. m. Group II, the control, received no lactose. On March 15, approximately 45,000 sporulated oöcysts were introduced into the crop of each bird in both groups. Cecal droppings for pH determinations were collected twice each day. The pH determinations of the cecal droppings and the effect of the inoculation on the chicks are recorded in table 1.

The pH determinations of the cecal droppings showed a constant higher degree of acidity in the ceca of the birds of the lactose group than in the controls.

Blood appeared in the droppings of two birds (nos. 52 and 54) of the lactose group on the sixth day. No. 52 passed blood for one day only, but No. 54 continued to do so for three days. Merozoites were present in the bloody droppings from these birds. None of the birds in this group were otherwise visibly affected. Oöcysts were found in the droppings of all birds after the sixth day.

Three of the five controls were passing bloody droppings on the fifth day. All passed blood and three died from coccidiosis on the sixth day. The fourth death from this cause occurred on the ninth day. The one remaining bird ceased passing blood after three days and exhibited no further symptoms.

All the birds were killed for autopsy on the eleventh day. The ceca of four of those in the lactose group appeared to be normal. One cecum of No. 52, was filled with a caseous core.

Both ceca of the one survivor of the control group were filled with a bloody, caseous core.

The results indicate that the lactose feeding was of marked benefit in combatting artificial infection with sporulated oöcysts of *Eimeria avium*. Two of the birds in the lactose group passed bloody droppings in which merozoites were present, and oöcysts occurred in the cecal droppings of all birds. This is evidence that at least a part of the sporozoites released from the sporocysts were unharmed and invaded the cells of the cecal mucosa where both the sexual and asexual cycles of development were completed. It is possible that the dose of oöcysts was too large to be entirely overcome or that the increased acidity in the ceca was more destructive to the merozoites than to the sporozoites.

TABLE 2

pH of Cecal Droppings and Effect of Inoculation with Sporulated Oöcysts in the Second Trial

	Bird No.	Mar. 29		Mar. 30		Mar. 31		Apr. 1		Apr. 2		Apr. 3		Apr. 4		Apr. 5		Apr. 6		Apr. 7		Apr. 8
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.
Group I (Lactose)	61	5.6	5.2	5.4	X	5.4	X	5.2	5.4	5.2	5.0	5.6	5.2	5.4	5.4	5.6		B	B	B	—	X
	62	5.4	5.2	5.2	X	5.0	5.0	5.4	—	5.0	5.4	5.4	5.4	5.2	5.4	5.0	X	B	—	B	—	X
	63	5.2	5.4	5.2	X	5.2	5.2	5.2	5.6	5.6	5.2	5.4	5.4	5.4	5.6	5.4	X	B	B	B	—	X
	64	5.0	5.0	5.4	X	5.4	X	X	X	5.4	—	5.2	—	5.0	X	—	X	D	—			
	65	5.2	5.2	5.0	X	5.2	X	5.4	5.0	5.2	5.2		5.0	5.2	5.2	X	—	B	B	B	—	X
Group II (Controls)	66	6.4	—	6.6	—	5.4	—	6.2	—	6.6	—	6.4	6.0	6.0	B	B S	—	D				
	67	6.8	—	6.4	—	6.6	—	6.0	—	6.6	—	6.8	6.2	6.0	—	B S	—	D				
	68	6.8	—	6.2	—	5.8	—	6.6	6.4	—	—	6.0	—	—	B	B	—	B	B S	S		S**
	69	6.6	—	—	—	6.0	5.6	6.6	—	6.0	—	6.0	—	6.4	B	B S	—	B S	D			
	70	6.2	6.2	6.2	—	6.0	—	6.4	6.4	6.6	—	6.4	—	5.6	—	B S	—	B S	B S	S		S D*

S = Bird visibly sick.
B = Cecal droppings contain blood.
D = *Died from coccidiosis.*
X = Cecal droppings watery and mixed with other droppings.
— = No cecal droppings.
* = No. 70 died on Apr. 12.
** = No. 68 recovered.

Second Trial

Ten chicks, six weeks old, were divided into two groups of five. The method of procedure was the same as in the first trial. The feeding of lactose to the birds in Group I was started on March 28, 1924. Three days later, approximately 40,000 sporulated oöcysts were introduced into the crop of each bird of both groups. The pH determinations of the cecal droppings and observations on the effect of the inoculation are given in table 2.

As in preceding experiments, an abnormal degree of acidity in the ceca was produced by the feeding of lactose.

One bird of the lactose group began voiding bloody droppings on the fifth day and three others on the sixth day. Both merozoites and oöcysts were found in the droppings. The appearance of the droppings had become normal on the eighth day and these birds exhibited no further symptoms. The fifth bird of Group I was found dead from coccidiosis on the morning of the sixth day. It had not previously appeared sick nor passed blood with the droppings.

Three of the control group were voiding bloody droppings on the fourth day. On the fifth day all showed marked droopiness and were passing blood. Four died from coccidiosis, three on the sixth day and one on the twelfth day. The remaining bird was visibly sick for several days but finally recovered in so far as the manifestation of symptoms was concerned.

All survivors were killed for autopsy on the fifteenth day after inoculation. The ceca and other organs of the four birds of the lactose group were normal in appearance. Microscopic examination of the cecal contents for coccidial cysts was negative.

The ceca of the one survivor of the control group were found to be entirely filled with solid, bloody, caseous cores which contained numerous oöcysts.

These results closely parallel those of the preceding trial. It was again demonstrated that feeding lactose did not prevent the sporozoites from invading the epithelial lining of the ceca where the cycles of development of the parasites were completed. In four of the five birds, however, further development of disease was arrested. The death of the one bird might have resulted entirely from the tissue damage caused by the invasion of the sporozoites and completion of their developmental cycles. This would appear to be additional evidence that the acidity produced in the ceca by feeding lactose to chickens is more destructive to the merozoite than to the sporozoite forms of ***Eimeria avium.***

TABLE 3

pH of Cecal Droppings of Birds in the Third Trial

	Cage No.	Mash fed	Number of birds	May 21 A.M.	May 21 P.M.	May 22 A.M.	May 22 P.M.	May 23 A.M.	May 23 P.M.	May 24 A.M.	May 24 P.M.	May 25 A.M.	May 25 P.M.	May 26 A.M.	May 26 P.M.	May 27 A.M.	May 27 P.M.	May 28 A.M.	May 28 P.M.	May 29 A.M.	May 29 P.M.
Group I (control)	1	Plain Mash	4	7.2	7.4	7.4	7.2	6.8	7.2	7.4		B	B	B	—						
	2		4	7.2	7.0	7.0	6.8	7.0	6.8	7.4		B	B	B	—						
	3		4	7.4	7.0	7.2	7.0	6.8	6.8	6.8		B	B	B	—	No cecal droppings passed after May 26.					
	4		3	7.4	6.8	7.4	6.8	7.2	6.8	7.0		B	B	B	—						
	5		3	7.0	7.2	7.2	7.0	7.0	7.0	6.8		B	B	B	—						
	6		2	7.6	6.2	6.8	7.2	7.4	6.8	7.2		B	B	B	—						
Group II	7	40% skim milk powder	3	5.6	X	6.8	5.6	5.8	5.4	6.0	No P.M. samples taken	6.0 B	—	5.4 B	5.2	5.4	—	5.4	—	5.2	—
	8		2	6.8	5.6	5.2	5.2	5.2	5.4	5.6		5.8 B	—	5.2 B	—	B	—	—	—	5.2	—
										5.8											
	9		3	5.4	5.4	5.4	5.4	5.0	5.2	7.0		5.0 B	—	5.4 B	—	5.4 B	—	5.4	—	5.4	—
										6.8											
	10		4	5.0	5.2	5.6	5.2	5.4	5.2	5.2		4.8 B	—	5.0 B	—	5.2 B	—	5.2	—	5.2	—
														5.4							
	11		3	5.2	5.4	5.4	5.4	5.2	5.6	5.4		5.2 B	—	B	—	5.2 B	—	5.2	—		
	12		2	6.2	X	5.2	5.4	5.0	5.4	5.6		5.0 B	—	B	—	—	—	—	—	5.4	—
										5.2											
Group III	13	20% Lactose Mash	1	5.4	5.2	5.6	5.2	5.2	5.6	5.2		5.0	—	B	—	5.2 B	—	—	—	5.2	5.0
	16		4	6.6	5.6	5.4	5.0	5.0	5.4	5.2		5.4 B	—	B	—	5.2 B	—	—	—	5.2	5.0
										5.4											
	14		4	5.4	X	5.4	5.2	6.2	5.4	5.8		5.6 B	—	B	—	—	—	—	—	5.2	—
	17		4	X	5.2	5.4	5.6	5.0	5.2	5.0		5.0 B	—	5.0 B	—	5.0	—	—	—	5.2	—
										4.8											
	15		4	5.6	5.4	7.0	5.0	7.0	5.4	5.8		5.0 B		B	—	—	—	—	—	5.2	—
	18		1	X	5.4	5.2	5.2	5.2	5.2	5.4		B		All birds dead.							

B = Droppings contain blood. When "B" alone appears in a space, it indicates that all cecal droppings contained blood and no normal sample was taken for pH determination. When both B and a pH value appear in a space, it indicates that blood was present in some of the cecal droppings but some normal droppings were present and were sampled.

— = Cecal droppings absent or liquid and mixed with other droppings.

THIRD TRIAL

In this experiment, the protection of chicks against coccidiosis was attempted by feeding them lactose or dry skim milk mixed with their mash. Sixty-eight chicks, six weeks old, were used. On May 20, approximately 50,000 sporulated oöcysts were introduced into the crop of each bird. The chicks were divided into three groups and immedately supplied wth the following rations:

Group I (controls), consisting of twenty-four birds, was fed a plain mash mixture.

Group II, consisting of twenty-two birds, was fed mash containing 40 per cent dry skim milk.

Group III, consisting of twenty-two birds, was fed mash containing 20 per cent lactose.

The dry skim milk contained 50.6 per cent lactose. This made the lactose content of the mashes for groups II and III approximately the same.

Fourteen deaths from chilling occurred during the three days following infection. The number of birds in Group I was thus reduced to twenty and in Groups II and III to seventeen each.

Mash was kept constantly before the birds, no other food being supplied. No preliminary feeding of dry skim milk or lactose before the administration of oöcysts was given.

From two to four birds were placed in each cage. Samples of cecal droppings for pH determinations were collected twice daily. Since there was more than one bird in a cage, it could not be determined from which individual a particular sample originated. This procedure, however, served to show differences between the pH values of cecal droppings of the three groups. When all deposits of cecal droppings from the birds in a cage were of the same physical character, one sample only was taken, otherwise more than one sample was taken. The first samples of droppings were collected on May 21, the day after the birds were inoculated and the feeding of lactose and dry skim milk was begun. The pH determinations and a summary of the effect of the inoculation on the birds are given in tables 3 and 4.

With few exceptions, the pH of the cecal droppings from the birds of Groups II and III was between 4.8 and 5.6, while in Group I, it ranged from 6.2 to 7.4.

A portion of the cecal droppings passed by Groups II and III on the fifth day contained some blood. This was more marked on the sixth day. A slight amount of blood was present in the droppings on the seventh and eighth days, but thereafter they were normal.

TABLE 4

EFFECT OF INOCULATION WITH SPORULATED OÖCYSTS IN THE THIRD TRIAL

Group No.	Mash fed	Number of birds	5th day*	6th day	7th day	8th day	9th day	Total died from coccidiosis	Per cent died from coccidiosis
I Controls	Plain	20	4 B 8 S 3 D	4 B 8 S 8 D	X 2 S 6 D	X 2 S 1 D	X 0 S 0 D	18	90
II	40% Dry skim milk	17	2 B 1 S 2 D	3 B 4 S 6 D	2 B 0 S 2 D	1 B 0 S 0 D	0 B 0 S 0 D	10	58.8
III	20% Lactose	17	2 B 2 S 3 D	3 B 2 S 7 D	2 B 0 S 1 D	0 B 0 S 0 D	0 B 0 S 0 D	11	64.7

D=Died from coccidiosis. Numeral preceding indicates number of birds.
S=General symptoms such as droopiness, inappetence. Numeral preceding indicates number of birds.
1 B=Blood present in less than half of cecal droppings.
2 B=Blood present in more than half of cecal droppings, but not in all.
3 B=Blood present in all cecal droppings, but all droppings not entirely blood.
4 B=*Cecal droppings appear to be entirely blood.*
X=No cecal droppings passed.
*=No indications of coccidiosis before fifth day.

Blood was discharged profusely from all birds in Group I on the fifth and sixth days. No cecal droppings were passed by these birds after the sixth day.

As shown in table 4, the first deaths from coccidiosis in all three groups occurred on the fifth day. The total mortality from this cause was eighteen, or 90 per cent, in Group I; ten, or 58.8 per cent, in Group II; and eleven, or 64.7 per cent, in Group III.

Besides suffering a lower mortality than the controls, a smaller number of the birds that were fed dry skim milk or lactose exhibited general symptoms and their droppings contained less blood.

The mortality from coccidiosis was relatively high in all groups. This may have been influenced by a chilling the birds received during the first two nights after the experiment was begun and also by the large dose of oöcysts they received. The results demonstrate, however, that feeding chicks mash containing 40 per cent dry skim milk, or 20 per cent lactose, is of considerable benefit in protecting them against artificial infection with sporulated oöcysts of *Eimeria avium.*

Fourth Trial

This experiment was designed to show the value of feeding of dry skim milk or lactose in protecting chicks, kept under conditions approximating those found in the field, against artificial infection with sporulated oöcysts of *Eimeria avium*.

Day-old chicks from a commercial hatchery were transferred directly to clean brooder pens. To avoid natural infection with coccidiosis, no outside runs were provided.

The chicks were divided into three groups of two pens each. To Group I was fed the following mash mixture:

Dry skim milk	40 parts
Wheat bran	10 parts
Yellow corn meal	30 parts
Ground barley	20 parts
Cod-liver oil	2 parts

The mash mixture for Group II consisted of:

Lactose	20 parts
Wheat bran	30 parts
Bone meal	10 parts
Meat scrap	15 parts
Ground barley	15 parts
Yellow corn meal	10 parts
Cod-liver oil	2 parts

For Group III, the controls, the mash was:

Wheat bran	20 parts
Bone meal	5 parts
Meat scrap	15 parts
Yellow corn meal	30 parts
Ground barley	30 parts
Cod-liver oil	2 parts

At the time these experiments were in progress there was no supply of green feed available in our location which was known to be free from contamination with oöcysts, therefore, cod-liver oil was included in the mash to supply vitamin A.

Scratch grain fed to all pens consisted of equal parts of fine cracked yellow corn, steel cut oats, and cracked wheat.

The mash and grain were fed in the proportion of two parts of mash to one part of grain. Fed in this proportion, the nutritive ratio of the

TABLE 5

TABULATED SUMMARY OF FOURTH TRIAL

Group No.	Pen No.	Number of chicks March 5	Mash fed	Method of inoculation	Died from coccidiosis						
					6th day	7th day	8th day	12th day	13th day*	Total	Per cent
I	1	19	Milk powder	250,000 oocysts orally	2	4	2	None	None	8	42.1
II	1	18	Lactose	250,000 oocysts orally	7	5	2	None	None	14	77.7
III	1	25	Plain (control)	250,000 oocysts orally	16	6	None	1	None	23	92
I	2	24	Milk powder	12,500,000 oocysts in soil	None	None	None	None	None	None	None
II	2	23	Lactose	12,500,000 oocysts in soil	1	3	1	None	None	5	21.7
III	2	24	Plain (control)	12,500,000 oocysts in soil	None	None	None	None	1	1	4.1

* No deaths occurred after the thirteenth day.

rations for all groups was 1 to 3.0.* Mash in metal hoppers was kept before the chicks continuously. The amount of mash consumed was determined by placing a weighed amount in the hoppers each morning and weighing out the unconsumed portion on the following morning. The difference in the weights represented the amount consumed during the preceding twenty-four-hour period and served as an index of the amount of grain to feed to preserve the two to one mash and grain ratio.

When the chicks were fourteen days old, inoculations with sporulated oöcysts were made as follows: Approximately 250,000 oöcysts were introduced into the crop of each chick in Pen I of all three groups. In Pen 2 of all three groups was placed a box of sterilized soil to which was added approximately 12,500,000 oöcysts. The grain fed in these pens thereafter was scattered on the soil. It was thought that by this means the chicks could be made to acquire coccidial infection in a more natural manner.

At the time the oöcysts were administered, the number of chicks in the different pens was as follows:

Group I—Pen 1 contained 19 chicks; Pen 2, 24 chicks
Group II—Pen 1 contained 18 chicks; Pen 2, 23 chicks
Group III—Pen 1 contained 25 chicks; Pen 2, 24 chicks

The oöcysts were administered on March 5. The chicks were kept under observation until March 31.

Deaths occurred from coccidiosis on the sixth day after inoculation and continued until March 18, the thirteenth day. There was no sickness nor death after that date. A tabulated summary of results is given in table 5.

In this table, it is seen that the total mortality of birds receiving the oral administration of oöcysts was 92 per cent in the control pen, 77.7 per cent in the lactose pen, and 42.1 per cent in the dry skim milk pen. Such relatively high mortality in all pens is not greater than is to be expected in view of the enormous dose of oöcysts given to each chick. The results demonstrate, however, that feeding chicks mash containing 40 per cent of dry skim milk affords them considerable protection against severe coccidial infection.

The results obtained with lactose were much less satisfactory. The results of preceding experiments indicate that the value of dry skim milk for coccidiosis control lies in the lactose it contains. Therefore, mashes containing 20 per cent lactose and 40 per cent dry skim

* The writers are indebted to W. E. Newlon for assistance in compounding the rations.

milk should be equally effective. A possible explanation of the failure of lactose mash in this case is that a sufficient quantity was not consumed. It was observed that much of this mash was scattered in the litter about the feed hopper, while the dry skim milk mash was consumed without waste.

The difference in the character of the two mashes appeared to be the factor responsible for the waste in one case and lack of waste in the other. The lactose mash contained 30 per cent of bran and 15 per cent of meat scrap. This made a coarse, flaky mixture that was easily scratched out of the hopper. The coarse brown particles of meat scrap appeared to be attractive to the chicks and in their efforts to pick them out the lighter part of the mash was thrown out on the floor and lost. The dry skim milk mash, on the other hand, was a uniform, nearly white, somewhat adhesive mixture of fine texture which did not tempt the chicks to pick through it and which was, therefore, consumed without waste.

The only serious mortality resulting from feeding grain on soil with which oöcysts had been mixed was that of 21.7 per cent in the lactose pen. No chicks died in the dry skim milk pen and only 4.1 per cent died in the control pen. The explanation of the slight degree of infection among the control chicks is evidently the failure on their part to ingest enough oöcysts to produce disease, since it is definitely known that the oöcysts were present in the soil and that the chicks were susceptible.

Fifth Trial

The method of procedure followed in this experiment, with the exception of the changes noted below, was the same as in the immedately preceding one. Pens in which infecton was attempted by means of feeding grain in soil contaminated with sporulated oöcysts were omitted because of the uncertainty of infecting chicks by this method. The mash mixtures were the same, except that wheat shorts were substituted for wheat bran and the meat scrap was sifted through a fine screen. The purpose of these changes was to provide as nearly as possible the same degree of fineness in all mashes. It was thought that by this means the temptation for the chicks to pick over the mash would be removed and the wasting of mash from this cause thereby avoided.

One hundred and fifty chicks, forty-eight hours old, were divided into three pens of fifty chicks each. They were given their first feed when seventy-two hours old. Pen 1 received the 40 per cent skim

milk powder mash; pen 2, the 20 per cent lactose mash, and pen 3, the controls, the plain mash.

During the first five days, the ration consisted entirely of mash which was before the chicks at all times. At this point, the sudden onset of a period of cold, damp weather had an unfavorable effect on all of the chicks, but was more serious among those in Pens 1 and 2 than in the control pen. This appeared to be due to the fact that the litter in Pens 1 and 2 became damp, while that in Pen 3 remained dry. This dampness resulted from the watery consistency of the droppings from the chicks fed dry skim milk and lactose. It was thought desirable, therefore, to reduce consumption of these mashes until the weather moderated by feeding scratch grain in addition to the mash. Scratch grain was fed twice daily for ten days. The chicks in all pens now appeared equally vigorous. From this time until the termination of the experiment, grain was fed in the morning only and the amount supplied restricted to one-third that of the mash consumed.

On April 30, when the chicks were eighteen days old, 1000 sporulated oöcysts were introduced into the crop of each chick. Pen 1 now contained forty-two chicks; Pen 2, thirty-nine chicks; and Pen 3, forty-two chicks.

Deaths from coccidiosis began on the sixth day after inoculation and continued through the seventh and eighth days.

A summary of the results is given in table 6:

TABLE 6

TABULATED SUMMARY OF FIFTH TRIAL

Pen No.	Ration	Number of chicks inoculated	Total died from coccidiosis	Per cent died from coccidiosis	Average weight per chick 32 days old	Average daily mash consumption per chick
1	Skim milk powder mash	42	1	2.3	185.5 gm.	12.5 gm.
2	Lactose mash	39	3	7.7	133.3 gm.	10.9 gm.
3	Plain mash (control)	42	10	23.8	146.9 gm.	12.9 gm.

The results, as shown in this table, clearly demonstrate the effectiveness of dry skim milk in combatting coccidial infection. Bloody droppings were passed by several birds in Group 1 in addition to the one which died, but none of them were visibly sick.

The results obtained with lactose were less satisfactory than those with dry skim-milk, but still demonstrated that the birds to which it

was fed were given considerable protection against coccidiosis. As recorded in the table, it was found that the mash consumption in this pen was less than in either of the other two. This is probably a factor responsible for the difference in the effectiveness against coccidiosis afforded by the dry skim milk and lactose mash mixtures.

Another factor that is probably in part responsible for the greater effectiveness of dry skim milk against coccidiosis is the superior food value of this milk product as indicated by the increased growth made by the chicks in Group 1.

This increase in weight amounted to 38.6 grams a chick, or 26.2 per cent more than was made by those in the control pen, which were fed the plain mash, in spite of the fact that the latter consumed 0.4 grams more mash per chick daily than those receiving the dry skim milk mash. The chicks which were fed the lactose mash consumed 12.8 per cent less than those receiving dry skim milk mash and, therefore, attained the least growth.

Summary and Conclusions

The results of the series of five experiments were uniform in demonstrating that chicks were afforded a considerable degree of protection against coccidial infection when a sufficient amount of lactose or dry skim milk was added to their diet. In the trials carried out under laboratory conditions, this was accomplished equally well by the individual administration of two 1-gram doses of lactose to each bird daily at an interval of about eight hours or by feeding chicks continuously with mash containing 20 per cent lactose or 40 per cent dry skim milk. In the trials carried out under field conditions, however, the results obtained from the use of skim-milk powder were superior to those obtained from the use of lactose. This was due, at least in part, to the fact that the chicks did not relish the mash mixture containing lactose and, therefore, consumed less of this mash than of that containing dry skim milk. The relatively greater increase in weight of the chicks fed on dry skim milk indicated that the superior food value of this material was also at least in part responsible for the benefit derived from its use.

The results of these experiments, confirm those described in the preceding paper in showing that when sufficient lactose or dry skim milk is fed to chickens, the hydrogen ion concentration of the cecal contents can be kept within a range of 4.4 to 5.6. It is thought that this degree of acidity may be sufficient to injure or destroy the sporo-

zoite or merozoite forms of *Eimeria avium* and that serious harm from the infection is thereby prevented. However, both merozoites and oöcysts were found in the droppings of birds inoculated with sporulated oöcysts and treated with lactose or dry skim milk even though the birds showed no visible signs of sickness after the inoculation. This is evidence that at least a part of the sporozoites released from the sporocysts were unharmed and invaded the cells of the cecal mucosa where both the sexual and asexual cycles of development were completed. A possible explanation of this is that the dose of sporulated oöcysts given was too large to be entirely overcome and, therefore a portion of the sporozoites escaped. Another possible explanation is that the acidity in the ceca was more destructive to the merozoites than to the sporozoites. In such a case, the invasion of the epithelial cells by the sporozoites and the completion of the developmental cycles within the cells would be unhindered. The merozoites, however, upon emergence from the epithelial cells into the acid cecal contents would be destroyed and further development of disease arrested. On this basis, the appearance of blood in the droppings and death on the fifth and sixth days after inoculation of some of the birds which were fed lactose or skim-milk powder could be ascribed to the tissue damage resulting from the initial invasion with sporozoites. The destruction of the merozoites, however, prevented further development of disease in the birds which were not fatally injured by the sporozoite invasion.

This explanation would not apply to the failure of lactose feeding in the last two coccidiosis control trials, to afford the chicks as high a degree of protection against coccidial infection as was given by dry skim milk. This, as previously pointed out, was probably due in part to the difference in amount of consumption of the two mash mixtures by the chicks (12.8 per cent less of lactose) and also in part to the superior food value of the skim-milk powder.

The fact that feeding chickens mash containing 40 per cent dry skim milk not only protected them against coccidial infection, but also stimulated rapid growth indicates that this would be a valuable practice in the prevention and control of outbeaks of the disease on poultry farms.